Investigating SNPs in the ZC4H2, FGD1, and MCT8 Genes in Humans

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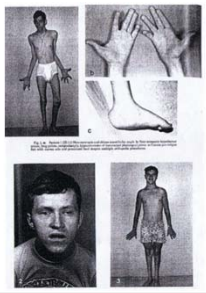
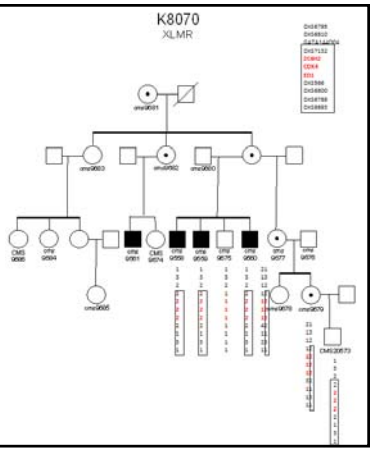
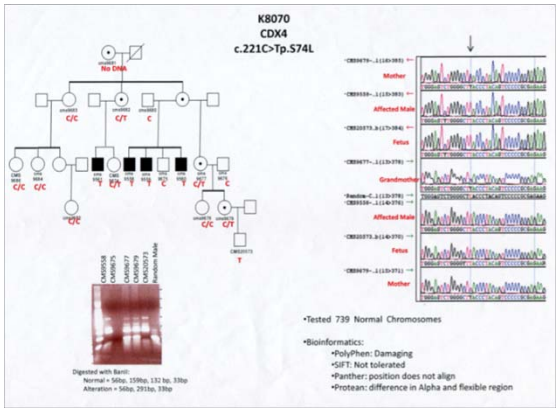
K8070



- Three genes in linkage region
  - ZC4H2
  - CDX4
  - ED1

- Severe Mental Retardation
- Postnatal Retardation of growth
- Facial Features:
  - Midface hypoplasia
  - Medial eyebrow flare
  - Exotropia +/- ptosis
  - Obtuse mandibular angle
  - High narrow palate
  - Open mouth/drooling
- Hands
  - Camptodactyly of fifth finger
  - Hyperextensible metacarpal phalangal joints
  - Long hands
  - Long palm
  - Syndactyly-soft tissue
  - Proximally placed thumbs
- Sidney Line
- Feet and Legs
  - Hallux valgus
  - Hypoplastic fifth nail
  - Open hallucal pattern
  - Distal muscle wasting
- Orthopedic-Other
  - Thoracic scoliosis
- Exotropia
- Microcephaly
- Low fintertip arches

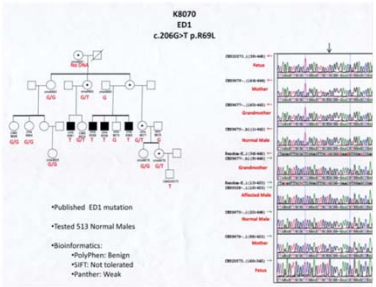




CDX4 EST Profile

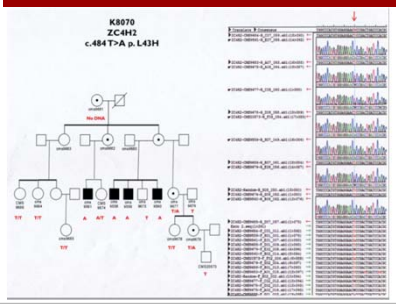
- No human EST profile
- Mouse Profile shows no expression of this gene in the brain

ED1 Profile

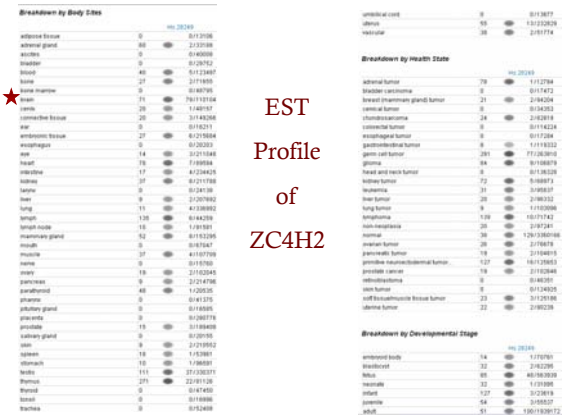


- Phenotype associated with this mutation does not match

ZCH42 Profile



- Confirmed by the Solids machine at GGC
- Conserved in chimpanzee, dog, cow, mouse, rat, chicken, zebrafish, fruit fly, mosquito



EST Profile of ZC4H2

ZC4H2 Bioinformatics

- A Zinc finger gene, c.484T>A in Exon 1
  - Polyphen: damaging
  - SIFT: tolerated
  - ipTree: destabilizing

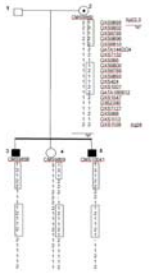
ZC4H2 Polymorphism Study

Plate	Patients that Amplified	Mutation Band present
Random 1 Lander Students	71	0
Random 3 UPD Males	43	0
SCS Black Males 1	76	0
Random 4 CEPH Males	58	0
Random Female Plate 1	48	0
Random Female Plate 2	84	0
Random Female Plate 3	132	0
Random Female Plate 4	118	0
Random Female Plate 5	122	0
Random Female Plate 6	68	0
Lander Female Plate 1	122	0
Lander Female Plate 2	180	0
SCS Black Females	180	0
Total:	**1302**	0

FGD1

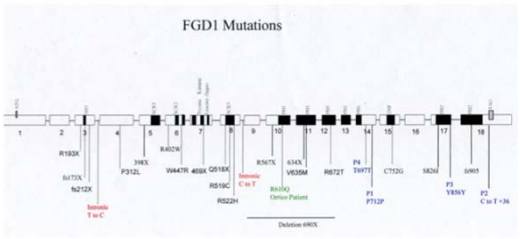
- Looking at family K9195
- Defects in this gene are the cause of faciogenital dysplasia/Aarskog-Scott syndrome and XLID
- Short stature, hypertelorism, shawl scrotum, brachydactyly

FGD1



- Exon 1
  - c.81 A>G p.H94R
- Gene encodes a protein that binds to a GTPase
- This protein also stimulates a mitogen activated protein kinase cascade

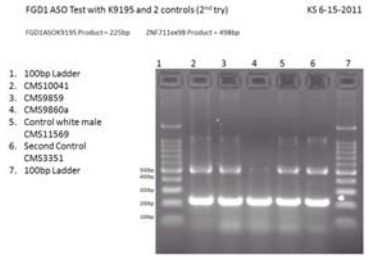
FGD1 Gene Map



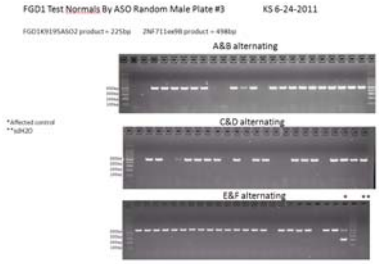
FGD1 Bioinformatics

Protein Website	Amino Acid Change in Protein	Effect
IPTREE-STAB	H94R	Negative(destabilizing)
Mupro	H94R	Delta delta G = increase stability
I-Mutant2.0 (gpcr)	H94R	Decrease stability
Panther	H94R	Position does not align to the HMM
PolyPhen	H94R	Benign

We turned this . . .



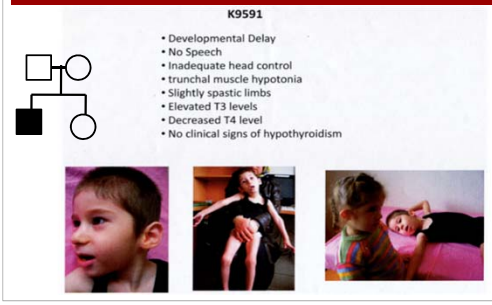
. . . Into this!
(with a new primer)



FGD1 Polymorphism Study

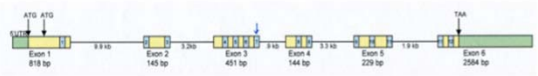
Plate	Patients that Amplified	Mutation Band present
Random Male 1	82	0
Random Male 3	56	0
Random Male 4	57	0
Random Male 6	14	0
Random Female 1	140	0
Random Female 2	158	0
Random Female 3	176	0
Random Female 4	188	0
Random Female 5	186	0
Total:	**1057**	0

Allan-Herndon-Dudley Syndrome

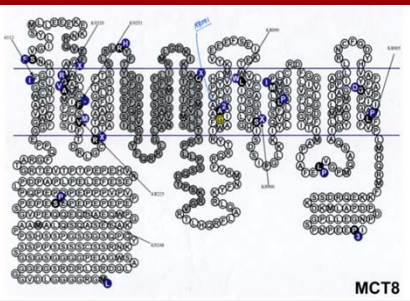


MCT8- Monocarboxylate Transporter 8

- 7th Transmembrane Domain
- Very active and specific thyroid hormone transporter
- Exon 3
  - c.1201G>A p.G401R
  - Conserved in humans, pigs, dogs, etc.



MCT8 Protein Structure



MCT8 Conservation Across Species

MCT8
c.1201G>A p.G401R
K9591

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Homo Sapiens                        MRVFRQRTYRIWAFGIAAAALGYFVPYV
Sus scrofa (pig)                    MRVFRQRTYRIWAFGIAAAALGYFVPYV
Canis familiaris (dog)              MRVFRQRTYRIWAFGIAAAALGYFVPYV
Pongo abelii (Sumatran orangutan)   MRVFRQRTYRIWAFGIAAAALGYFVPYV
Macaca mulatta (rhesus monkey)      MRVFRQRTYRIWAFGIAAAALGYFVPYV
Mus musculus (mouse)                MRVFRQRTYRIWAFGIAAAALGYFVPYV
Rattus norvegicus (rat)             MRVFRQRTYRIWAFGIAAAALGYFVPYV
Equus caballus (horse)              MRVFRQRTYRIWAFGIAAAALGYFVPYV
Bos taurus (cow)                    MRVFRQRTYRIWAFGIAAAALGYFVPYV
Ailuropoda melanoleuca (giant panda) MRVFRQRTYRIWAFGIAAAALGYFVPYV
Gallus gallus (chicken)             LRVFRRKTYRIWAFGIATAVLGYFVPYM
Taeniopygia guttata (zebra finch)   LRVFRRKTYRIWAFGIATAVLGYLVPYM
Drosophila persimilis               VEIWKRKRRFVIWALCVPLALFGYFVPYV
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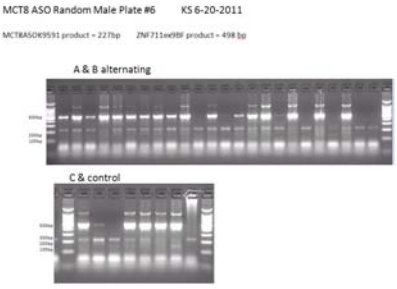
MCT8 Bioinformatics

Protein Website	Amino Acid Change in Protein	Effect
Panther	G401R	0.76208 probability of having a deleterious effect
PolyPhen	G401R	Probably damaging
IPTREE-STAB	G401R	Negative(destabilizing)
RESCUE-ESE	G401R	No discenernible affect
Mupro	G401R	Deltadelta G – increase stability
MUstab	G401R	Decreased protein stability, preditcion confidence = 81.61%
I-mutant	G401R	Decrease in stability



•After this gel, got all new buffer, water, etc. to eliminate possibility of contamination.
  •Still got bad results.
•Tried raising the temperature and adding more and less primer
•Nothing got rid of the mystery bands
  •Decided to sequence the samples for best accuracy

MCT8 Sequencing

Plate	Patients that Amplified	Base change present
Random Male 1	80	No
Random Male 3	54	No
Random Male 4	47	No
Random Female 1	172	No
Random Female 2	170	No
Random Female 3	186	No
Random Female 4	186	No
Random Female 5	178	No
Total:	**1073**	0